

## Colony PCR Protocol

This protocol is intended for use in testing the presence of particular plasmids in a colony of *E. Coli*. Two Controls are used, one utilizing the vector plasmid, the other being the plasmid inserted.

### Materials:

- Vector Plasmid
- Plasmid being inserted
- Q5 PCR Master Mix
- VF2 Primer
- VR Primer
- dH<sub>2</sub>O
- A supply of 0.5ml PCR tubes

### A single reaction requires these amounts:

15ul reactions -> 7.5ul master mix  
                          1ul 10mM VF2  
                          1ul 10mM VR  
                          5.5 ul dH<sub>2</sub>O

An example mega mixture for a 15 reactions (this includes the two controls)

112.5 ul master Mix  
1.5ul 100mM VF2  
1.5ul 100mM VR  
184.5ul dH<sub>2</sub>O

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Total volume ~300

From this mixture, insert 15ul into each tube.

### PCR Thermal Cycler Run

98C - 2min  
98C - 30sec ]  
55C - 30sec ] ---> x35 Cycles  
72C - 3min ]  
72C - 10min

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**Total Run Time ~ 2.5 Hours**